Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Highly sensitive multi-residue analysis of veterinary drugs including coccidiostats and anthelmintics in pond water using UHPLC-MS/MS: application to freshwater ponds in Flanders, Belgium.

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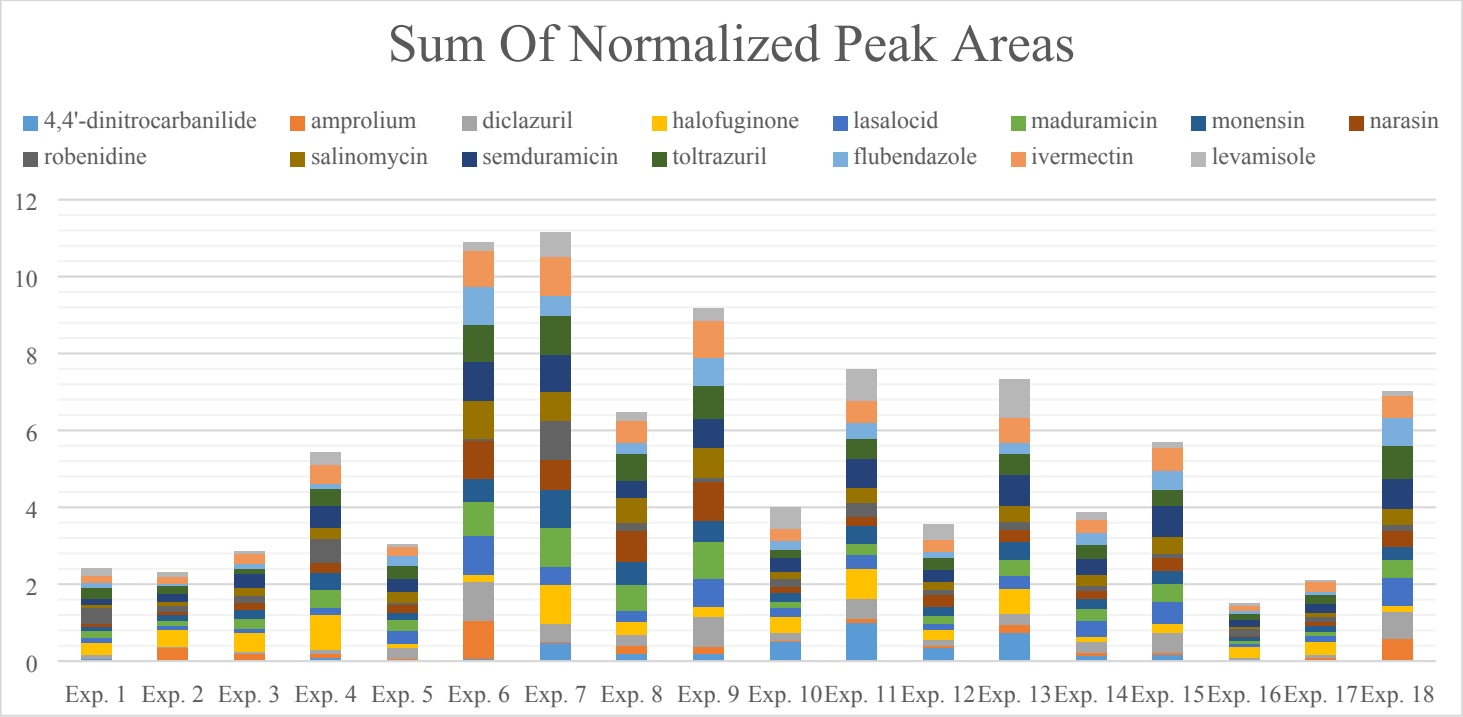


Figure S1. Sum of the normalized peak areas of the 15 veterinary drugs obtained for each experiment during extraction optimization. Experiments (n=18) were constructed using a statistical screening design (fractional factorial resolution or FRR), comprising different values for the selected extraction parameters. Experimental design was established using the software program JMP 12.0 (SAS institute Inc, Cary, USA).

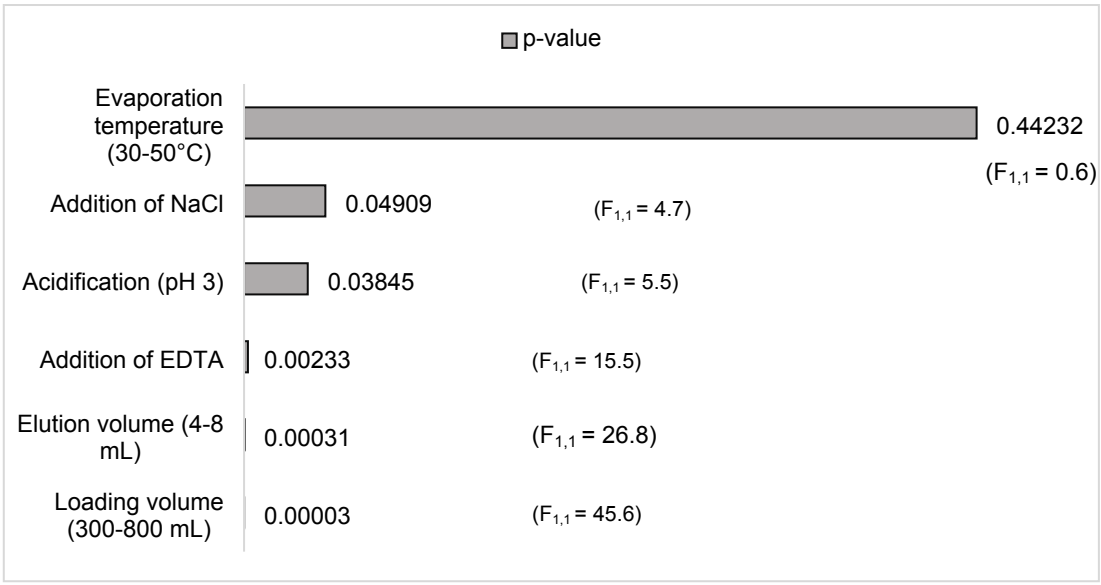


Figure S2. P-values of the selected extraction parameters evaluated using a fractional factorial resolution IV experimental design. P-values < 0.05 were retained for further investigation. The corresponding F-ratio for every parameter has been placed between brackets.

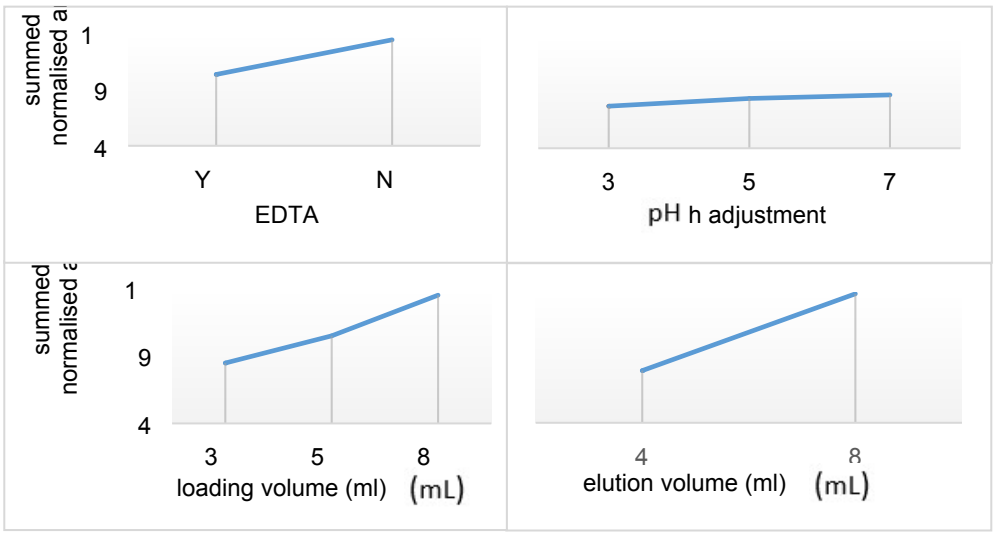


Figure S3. Optimization of the extraction parameters, evaluated using a fractional factorial resolution IV experimental design. Higher summed normalized areas were observed in the absence of EDTA in the sample, a sample pH of 7, higher loading volumes (>300 mL) and a higher elution volume (>4 mL).

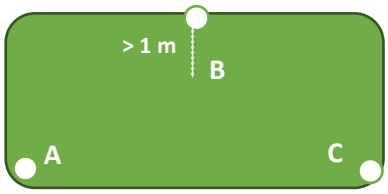
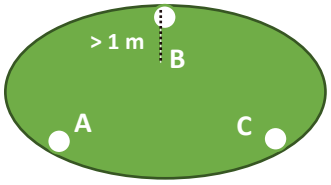
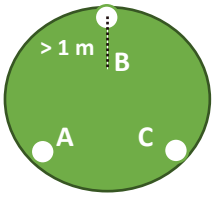
Pond shape	Sampling points		Ponds
Rectangular			BRA2a BRA3 ZOT5 ZOT6 GER1 MAARK2
Elliptic			BRA4 BRA6 ZOT1 ZOT4 ZOT7 ZOT8
Circular			BRA2b ZOT10 GER2 MAARK1 LIE ZOT9

Figure S4. Pond shapes of the 18 selected ponds including illustration of sampling points at three locations situated equidistantly from each other at <1m (A and C) and >1m (B) from shore.

Table S1. Physicochemical parameters, i.e. aqueous solubility, log P, pKa, half-life (DT_{50}) in water, organic carbon-water partition coefficient (K_{oc}) and vapor pressure of the 15 veterinary drugs included in the newly developed UHPLC-MS/MS method.

	Aqueous solubility	**Log P**	**pKa**	**DT_{50} in water**	**K_{OC}**	**vapor pressure**	**Reference**
	25°C, pH 7, mg L^{-1}	25°C	25°C, most acidic	25°C, pH 7, days	L kg^{-1}	25°C, mmHg	
Coccidiostats							
4,4'-dinitrocarbanilide	0.02	3.00	10.8	-	74,128	3.2×10^{-10}	Bampidis et al., 2018[1]
amprolium	540	1.04	4.7	-	1,928	4.9×10^{-15}	Rychen et al., 2018[2]
diclazuril	3.0	4.60	6.5	-	4,986	1.2×10^{-22}	Drugbank Database (www.drugbank.ca); Rychen et al., 2018[3]
halofuginone	114	1.24	14.5	14	-	-	Drugbank Database (www.drugbank.ca)
lasalocid	10	4.20	2.6	> 34	1,090	-	Bohn et al., 2013[4]
maduramicin	15.7	4.77	4.0	-	185	-	Drugbank Database (www.drugbank.ca)
monensin	63	4.82	4.2	> 34	460	5.2×10^{-23}	Bohn et al., 2013[4]; Bampidis et al., 2019 [5]
narasin	102	7.88	4.4	> 34	1,357	-	Bohn et al., 2013[4]; Bampidis et al., 2018[1]
robenidine	118	4.38	3.4	-	-	-	Bampidis et al., 2019[6]
salinomycin	294	7.51	4.4	> 34	389	-	Bohn et al., 2013[4]; Hansen et al., 2009[7]
semduramicin	30	4.20	3.9	-	1,116	-	Drugbank Database (www.drugbank.ca)
toltrazuril	1.04*	4.40	6.8	-	617	1.7×10^{-15}	Kim et al., 2010[8]; HPRA, 2017[9]
Anthelmintics							
flubendazole	1,000	3.40	9.2	7	1,1	-	Yoshimura, 2003[10]
ivermectin	4	5.83	12.5	30	3,981	1.5×10^{-9}	Drugbank Database (www.drugbank.ca); Liebig et al., 2010[11]
levamisole	1,440	2.36	6.9	30	-	-	Drugbank Database (www.drugbank.ca)

* = toltrazuril sulfone

- = not reported

Table S2. Experiments using a statistical screening design (fractional factorial resolution or FRR), comprising different values for the selected extraction parameters during method optimization. Design and results were established using the software program JMP 12.0 (SAS institute Inc, Cary, USA).

	Extraction Parameters				
	EDTA	NaCl	pH	Loading volume (mL)	Elution volume (mL)
Experiment 1	No	No	3	300	8
Experiment 2	No	No	3	500	4
Experiment 3	No	No	5	500	8
Experiment 4	No	No	5	800	4
Experiment 5	No	No	7	300	4
Experiment 6	No	No	7	800	8
Experiment 7	No	Yes	7	800	8
Experiment 8	No	Yes	5	300	4
Experiment 9	No	Yes	7	500	8
Experiment 10	Yes	No	3	500	4
Experiment 11	Yes	No	3	800	8
Experiment 12	Yes	No	5	300	8
Experiment 13	Yes	No	5	800	4
Experiment 14	Yes	No	7	300	8
Experiment 15	Yes	No	3	500	4
Experiment 16	Yes	Yes	3	300	4
Experiment 17	Yes	Yes	5	500	4
Experiment 18	Yes	Yes	7	800	8

Table S3. Protocol concerning the fortification of different calibrator samples applied in the multi-residue UHPLC-MS/MS method. WO_1, WO_2, WO_3 = mixed standard working solution 1, 2 and 3. IS = internal standard.

	Conc. in 500 mL sample (ng L^{-1})				**Applied mixed standard working solution**			**Applied mixed IS working solution**		
	Group 1[A]	Group 2[B]	Group 3[C]	Group 4[D]	Code	Conc. (µg mL^{-1})	spiked vol. (µL)	Code	Conc. (µg mL^{-1})	spiked vol. (µL)
Cal 1	0.25	2.5	2.0	12.5	WO_3	0.005[A], 0.05[B], 0.04[C], 0.250[D]	25	WO_{IS}	1	25
Cal 2	0.5	5.0	4.0	25	WO_3	0.005[A], 0.05[B], 0.04[C], 0.250[D]	50	WO_{IS}	1	25
Cal 3	1.0	10	8.0	50	WO_3	0.005[A], 0.05[B], 0.04[C], 0.250[D]	100	WO_{IS}	1	25
Cal 4	2.5	25	20	125	WO_2	0.05[A], 0.5[B], 0.4[C], 2.5[D]	25	WO_{IS}	1	25
Cal 5	5.0	50	40	250	WO_2	0.05[A], 0.5[B], 0.4[C], 2.5[D]	50	WO_{IS}	1	25
Cal 6	10	100	80	500	WO_2	0.05[A], 0.5[B], 0.4[C], 2.5[D]	100	WO_{IS}	1	25
Cal 7	25	250	200	1,250	WO_1	0.5[A], 5[B], 4[C], 25[D]	25	WO_{IS}	1	25
Cal 8	50	500	400	2,500	WO_1	0.5[A], 5[B], 4[C], 25[D]	50	WO_{IS}	1	25
Cal 9	100	1,000	800	5,000	WO_1	0.5[A], 5[B], 4[C], 25[D]	100	WO_{IS}	1	25
Cal 10	200	2,000	1,600	10,000	WO_1	0.5[A], 5[B], 4[C], 25[D]	200	WO_{IS}	1	25

[A] = amprolium, flubendazole and halofuginone

[B] = diclazuril, 4,4'-dinitrocarbanilide, maduramicin, monensin, narasin, robenidine, salinomycin, semduramicin and toltrazuril

[C] = lasalocid

[D]= ivermectin and levamisole

Table S4 Validation results of the within-day and between-day precision and apparent recovery experiments for the coccidiostats and anthelmintics included in the multi-residue UHPLC-MS/MS method.

	Within-Day (n=6)		Between-Day (n=3x3)	
	Precision	Apparent Recovery	Precision	Apparent Recovery
Coccidiostats	(RSD, %)	(%)	(RSD, %)	(%)
4,4'-dinitrocarbanilide				
2.5 ng L^{-1}	7.3	98.5	11.4	105.2
5 ng L^{-1}	5.0	95.7	5.1	100.2
50 ng L^{-1}	3.3	92.9	3.3	97.2
amprolium				
2.5 ng L^{-1}	3.4	99.1	5.9	101.4
5 ng L^{-1}	2.4	98.9	15.7	86.8
50 ng L^{-1}	12.8	87.7	12.0	90.1
diclazuril				
2.5 ng L^{-1}	7.2	105.8	15.3	102.8
5 ng L^{-1}	5.6	98.9	6.1	97.6
50 ng L^{-1}	2.5	89.8	7.0	93.7
halofuginone				
1 ng L^{-1}	15.8	90.7	21.1	85.8
5 ng L^{-1}	18.0	101.3	14.8	83.2
50 ng L^{-1}	8.7	104.8	22.8	88.9
lasalocid				
8 ng L^{-1}	13.3	99.1	6.0	103.9
20 ng L^{-1}	13.2	92.8	11.4	100.8
400 ng L^{-1}	17.5	86.4	22.9	112.6
maduramicin				
25 ng L^{-1}	16.9	73.4	8.6	88.5
50 ng L^{-1}	10.7	89.3	11.9	89.5
500 ng L^{-1}	8.9	107.7	16.9	91.4
monensin				
2.5 ng L^{-1}	14.2	100.5	17.7	91.6
50 ng L^{-1}	3.5	109.1	5.5	105.4
500 ng L^{-1}	7.5	102.8	6.6	101.4
narasin				
5 ng L^{-1}	11.1	97.3	9.8	107.0
50 ng L^{-1}	3.3	106.1	9.3	111.9
500 ng L^{-1}	4.4	103.6	8.0	110.7
robenidine				
25 ng L^{-1}	9.6	92.6	21.0	101.7
50 ng L^{-1}	19.6	95.1	30.9	95.9
500 ng L^{-1}	18.8	98.6	34.5	73.5
salinomycin				
2.5 ng L^{-1}	7.6	108.2	10.2	107.8

50 ng L^{-1}	4.4	107.2	4.7	101.7
500 ng L^{-1}	4.0	102.0	2.5	102.8
semduramicin				
10 ng L^{-1}	21.3	98.5	8.8	99.3
50 ng L^{-1}	14.1	80.0	7.6	86.8
500 ng L^{-1}	9.4	82.3	4.4	90.8
toltrazuril				
2.5 ng L^{-1}	12.0	112.8	13.2	112.2
50 ng L^{-1}	2.3	106.3	3.5	103.7
500 ng L^{-1}	2.8	96.6	3.8	95.0
Anthelmintics				
flubendazole				
2.5 ng L^{-1}	13.4	96.0	21.5	81.5
5 ng L^{-1}	14.6	105.3	9.4	98.8
50 ng L^{-1}	4.9	107.3	6.0	99.4
ivermectin				
125 ng L^{-1}	14.4	101.2	18.3	107.2
250 ng L^{-1}	8.7	105.8	19.7	115.8
2,500 ng L^{-1}	12.4	97.4	10.4	109.1
levamisole				
250 ng L^{-1}	6.6	95.9.6	4.2	100.8
2,500 ng L^{-1}	6.4	88.0	13.4	102.7

Table S5. Site locations of the sampled fresh water ponds situated in natural areas in Flanders, Belgium. Samples were used for the evaluation of matrix effects.

Sampling location	x coordinate	y coordinate	City
1	51.0250	3.4904	Destelbergen
2	51.0244	3.4917	Destelbergen
3	51.0151	3.4926	Destelbergen
4	51.0245	3.4927	Destelbergen
5	51.0313	3.4747	Destelbergen
6	51.0304	3.5004	Destelbergen
7	51.0259	3.5004	Destelbergen
8	53.0300	3.5001	Destelbergen
9	51.0250	3.5051	Laarne
10	51.0215	3.5008	Laarne
11	51.0923	3.2731	Maldegem
12	51.0911	3.2741	Maldegem
13	51.0904	3.2715	Maldegem
14	51.0915	3.2802	Maldegem
15	51.0922	3.2806	Maldegem
16	51.0924	3.2812	Maldegem
17	50.5648	3.4353	Merelbeke
18	50.5645	3.4348	Merelbeke
19	50.5656	3.4312	Merelbeke
20	50.5815	3.5943	Lede

Table S6. Site locations of the sampled fresh water ponds situated in 3 different geological regions in Flanders, Belgium. Samples were used to evaluate filtration efficiency and compound stability.

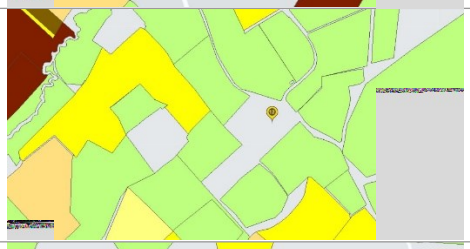
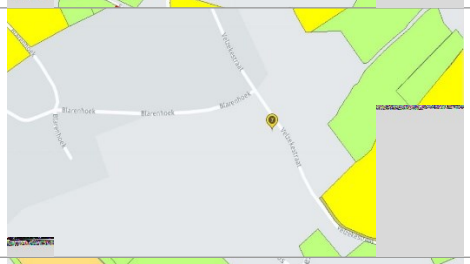
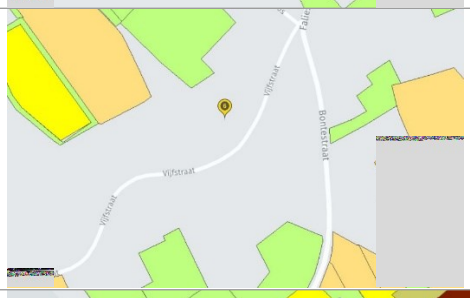
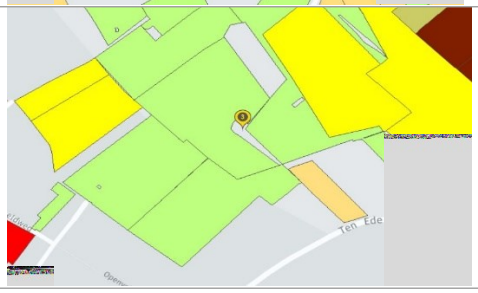
Sampling location	x coordinate	y coordinate	City	Soil Type
1	51.0924	3.2812	Maldegem	sand
2	50.5815	3.5943	Lede	loam
3	51.0316	3.4847	Destelbergen	heavy clay

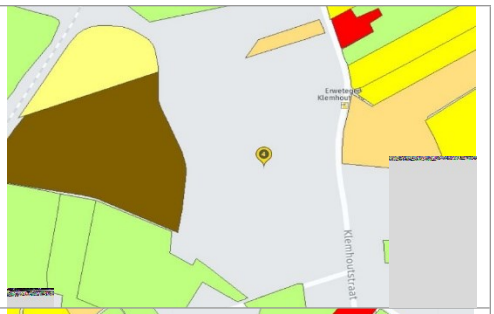
Table S7. Site locations and pH of the fresh water ponds in Flanders, Belgium, used for the environmental sample analysis.

Sampling location	x coordinate	y coordinate	City	pH
BRA2a	50.831628°	3.754453°	Brakel	7.72
BRA2b	50.831628°	3.754453°	Brakel	7.18
BRA3	50.793164°	3.718275°	Brakel	7.28
BRA4	50.791753°	3.726675°	Brakel	7.19
BRA6	50.783028°	3.727333°	Brakel	7.33
GER1	50.806296°	3.892352°	Geraardsbergen	8.04
GER2	50.808032°	3.887152°	Geraardsbergen	7.37
LIE	50.824792°	3.845023°	Lierde	7.03
MAARK1	50.785408°	3.662794°	Maarkedal	7.35
MAARK2	50.803242°	3.695028°	Maarkedal	8.31
ZOT1	50.880761°	3.794239°	Zottegem	8.00
ZOT10	50.824139°	3.800861°	Zottegem	6.86
ZOT4	50.835308°	3.799875°	Zottegem	8.23
ZOT5	50.837314°	3.820114°	Zottegem	7.18
ZOT6	50.838347°	3.830539°	Zottegem	7.18
ZOT7	50.838367°	3.842908°	Zottegem	8.11
ZOT8	50.834907°	3.824904°	Zottegem	7.55
ZOT9	50.826347°	3.792900°	Zottegem	7.65

Table S8. Pond location in relation to the surrounding agricultural land use (i.e. dominant cultivation and distance to nearby pasture) (Geopunt data source).

Pond location	Agricultural land use		Map of agricultural land use (Geopunt Land Cover Data 2018, LANDBGBRP)
	Agriculture	*Livestock production*	
	Dominant cultivation within 200 m	Distance to nearby pasture (m)	
BRA2a	potatoes	< 1	
BRA2b	potatoes	< 1	
BRA3	corn	75	
BRA4	cereals	< 1	
BRA6	corn	< 1	
GER1	cereals	< 1	

GER2	corn	33	
LIE	potatoes	96	
MAARK1	corn	38	
MAARK2	woody crops	< 1	
ZOT1	corn	89	
ZOT10	cereal	63	
ZOT4	corn	< 1	

ZOT5	sugar beets	140	
ZOT6	corn	20	
ZOT7	corn	< 1	
ZOT8	corn	37	
ZOT9	corn	110